

Figure 1

PG5 PTH(1-9)/(Gly)PTH(15-31)

1
A-V-S-E-I-Q-L-M-H-g-g-g-g-g-L-N-S-M-E-R-V-E-W-L-R-K-K-L-Q-D-V-NH2³¹

SEQ ID NO: 9

1 GCUGUUUCCG AAUCCAGCU GAUGCACGGU GGUGGUGGUG GUCUGAACUC

51 CAUGGAACGU GUUGAAUGGC UGCGUAAAAA ACUGCAGGAC GUU SEQ ID NO: 14

PG7 PTH(1-9)/(Gly)PTH(17-31)

1
A-V-S-E-I-Q-L-M-H-g-g-g-g-g-g-g-S-M-E-R-V-E-W-L-R-K-K-L-Q-D-V-NH2³¹

SEQ ID NO: 11

1 GCUGUUUCCG AAUCCAGCU GAUGCACGGU GGUGGUGGUG GUGGUGGUUC

51 CAUGGAACGU GUUGAAUGGC UGCGUAAAAA ACUGCAGGAC GUU SEQ ID NO: 15

PG9 PTH(1-5)/(Gly)PTH(15-31)

1
A-V-S-E-I-g-g-g-g-g-g-g-g-g-L-N-S-M-E-R-V-E-W-L-R-K-K-L-Q-D-V-NH2³¹

SEQ ID NO: 13

1 GCUGUUUCCG AAUCCGUGG UGGUGGUGGU GGUGGUGGUG GUCUGAACUC

51 CAUGGAACGU GUUGAAUGGC UGCGUAAAAA ACUGCAGGAC GUU SEQ ID NO: 16

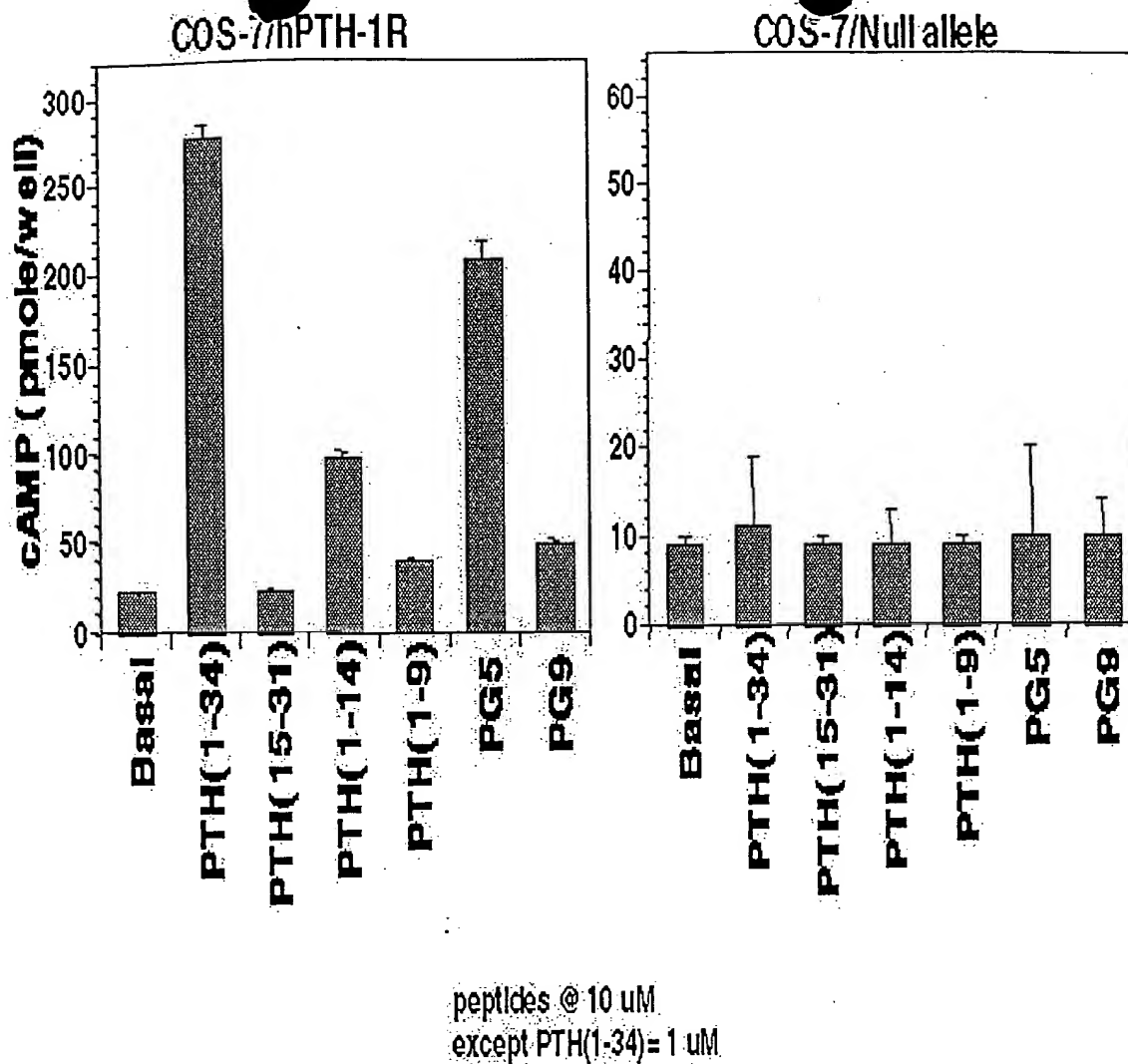
Family B Ligands

12-28-98

hpth	1	SVSEI QLMNLTCKH NSMERVENLR KTLQDVHNE	SEQ ID NO: 17
hpthrpi AVSEH	1	QLTHDKGKSI QDIRBREFFL HLAIEHTA	SEQ ID NO: 18
hpacapi HSDGI	1	FTDSYSRKR QMAVKYIAA VLGRKYKOR VNK	SEQ ID NO: 19
hw4P	1	HSDAV FTDNYTRLRK QMAVKYIINS ILN	SEQ ID NO: 20
hgze	1	XADAI FTNSYRKVLG QLSARKILOD IMSR	SEQ ID NO: 21
hphm	1	HADGV FTSDESKLLG QLSAKKYLES IM	SEQ ID NO: 22
hglpi	7	HAEGT FTSDEVSSYLE GQAKEFLAN LVKGRG	SEQ ID NO: 23
hglucagon	1	HSQGT FTSDESKYLD SRRAQDEVOM IMNT	SEQ ID NO: 24
gip	1	YAEGT FISDYSLAMD KIHODEVNM LLAQKGKN DPKHNITQ	SEQ ID NO: 25
hsecretin	1	HSDDT FTSLSRLRE GARLQRILQS IV	SEQ ID NO: 26
hcalcitonin	1	CGNLS TCMLGTYTOD ENKEHTFPQT AIGVGAP	SEQ ID NO: 27
hcgyp-2	1	ACNTA TCVTTRLAGL LSRSGGVMKS NEVPITNGSKAF	SEQ ID NO: 28
hcgrpi1 ACDTA	1	TCVTTRLAGL LSRSGGVVKN NEVPITNGSKAF	SEQ ID NO: 29
hamylln	1	KCNTA TCATQRIANF LVHSSNFGA ILSSTNVGSNTY	SEQ ID NO: 30
hadrenomedu	1	GCRFG TCTVOQLAHQ IYQETDKDK NVAPRSKISPO	SEQ ID NO: 31
horf	1	SEEPF ISDLTFELL REVELENARAB QLAQOASHNRKLMEII	SEQ ID NO: 32
sauvagine	1	EEBPI SIDLSLELR KMIEIEKQEK EKQOAHNNRLIDTI	SEQ ID NO: 33
msdh	1	TGAOS LISVAPLDVL RQRLMELNR RMRELQSGRIQQNRQLTISI	SEQ ID NO: 34
Maxadillin	1	CDAIC QERKAIDCQ KOAHHSNVLQ TSVOPTATFTSMDTSQLPGNVSFKECMKOKKEESSGK	SEQ ID NO: 35

Figure 3

A



B

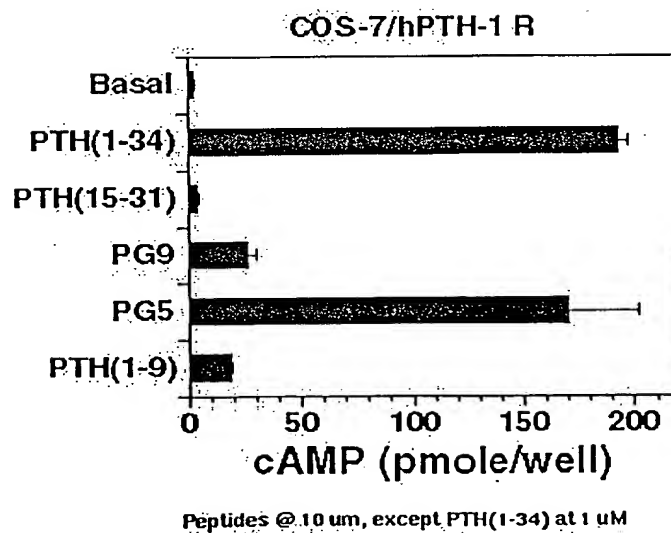
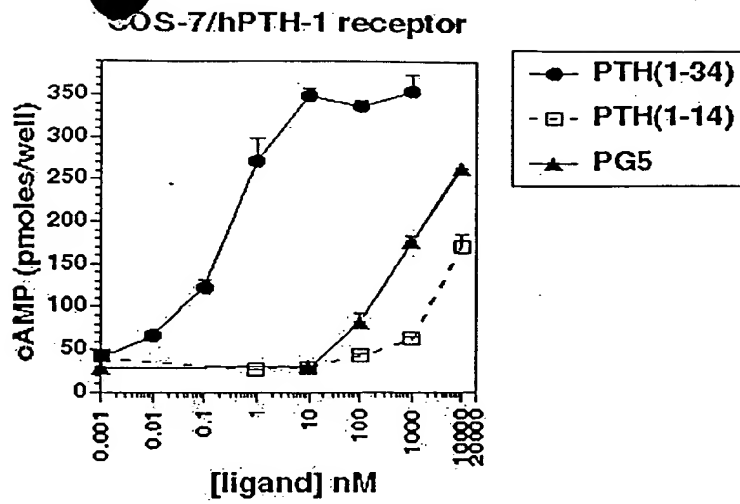
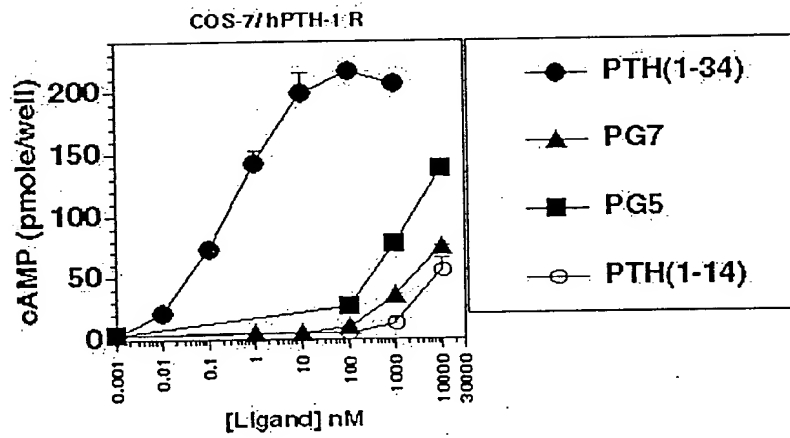


Figure 4

A



B



C

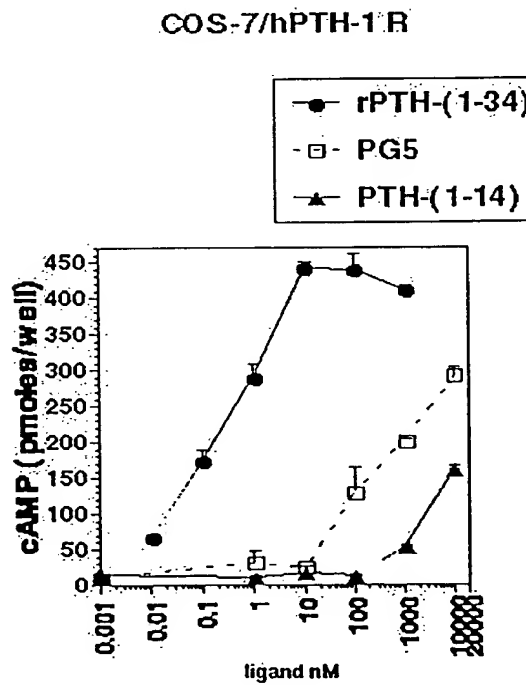


Figure 5

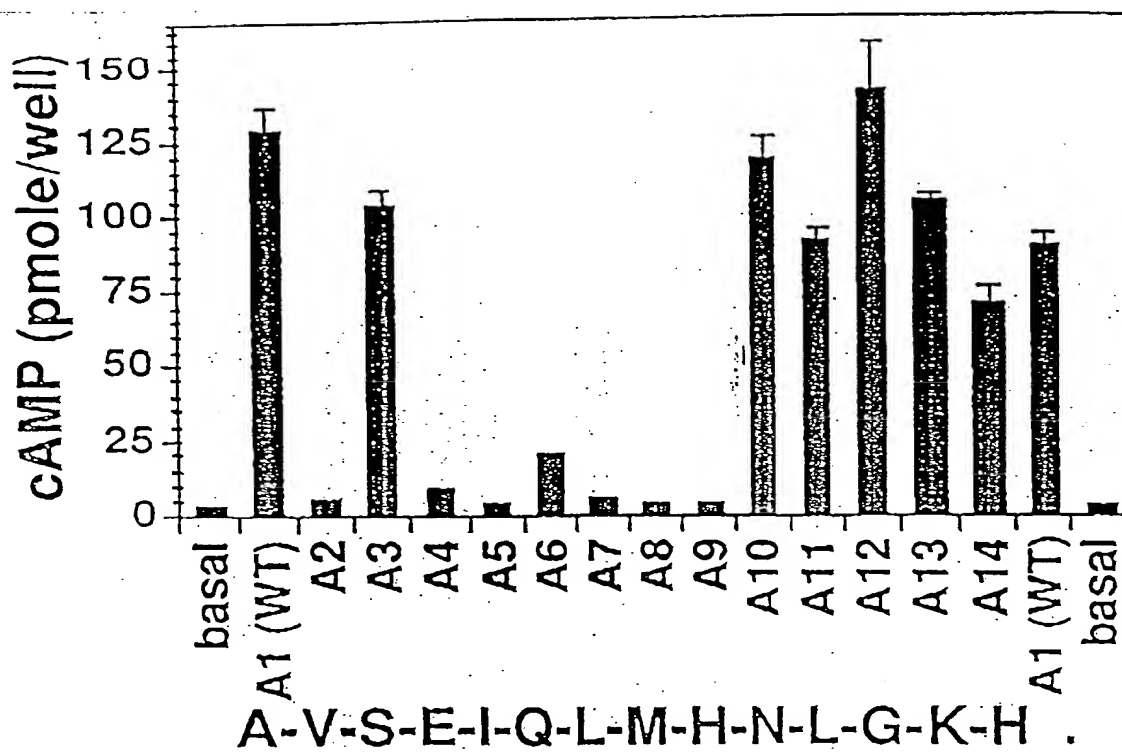


Figure 6

Alanine Scan of PTH(17-31)			
native PTH(17-31)		Binding IC ₅₀ (μ M) 1.0 ± 0.1	n 14
Position	Substitution		
17	Ser→Ala	1.5 ± 0.2	14
18	Met→Ala	1.5 ± 0.3	5
19	Glu→Ala	0.7 ± 0.1	5
20	Arg→Ala	90.5 ± 50.0	5
21	Val→Ala	1.7 ± 0.2	5
22	Glu→Ala	0.6 ± 0.2	8
23	Trp→Ala	> 100	8
24	Leu→Ala	67.5 ± 14.3	5
25	Arg→Ala	3.8 ± 0.9	5
26	Lys→Ala	8.3 ± 1.2	5
27	Lys→Ala	1.1 ± 0.1	5
28	Leu→Ala	9.9 ± 1.4	5
29	Gln→Ala	0.9 ± 0.1	5
30	Asp→Ala	1.1 ± 0.2	5
31	Val→Ala	3.8 ± 0.6	5
<p>Competition binding analysis for each PTH(17-31) peptide analog was performed in COS-7 cells transfected with PTH-2 receptors. IC₅₀ is the dose of a peptide analog which inhibited by 50% the binding of ¹²⁵I-rPTH(1-34). Each analysis was performed the number of times indicated(n).</p>			

Figure 7

MAP of: tether-1.seq from: 2319 to: 3698
 PTH(1-9) linked to Glu-182 of rat receptor. insert immediately after
 Tyr23 cleavage site. December 29, 1998 15:27 ..

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ATGGGGGGCCGCCGGATCGCACCCAGCCTGGCGCTCCTACTCTGCTGCCAGTGCTCAGC
M G A A R I A P S L A L L L C C P V L S
TCCGCcTATGCGGUUCCGAAUCCAGCUGAUGCACggcggaggaggcGAGGTATTTGAC
S A Y A V S E I Q L M H G G G G E V F D
CGCCTAGGCATGATCTACACCGTGGGATACTCCATGTCTCTCGCCTCCCTCACGGTGGCT
R L G M I Y T V G Y S M S L A S L T V A
GTGCTCATCCTGGCCTATTTTAGCGGGCTGCACTGCACGCGCAACTACATCCACATGCAC
V L I L A Y F R R L H C T R N Y I H M H
ATGTTCTGTCTGTTTATGCTGCGCGCCGAGCATCTTCGTGAAGGACGCTGTGCTCTAC
M F L S F M L R A A S I F V K D A V L Y
TCTGCTTCACGCTGGATGAGGCGGAGCGCTCACAGAGGAAGAGTTGCACATCATCGCG
S G F T L A Y F R R L T E E E L H I I A
CAGGTGCCACCTCCGCGCGCGCGCTGCCGCGTAGGCTACGCTGGCTGCCGCGTGGCGGTG
Q V P P P P A A A A V G Y A G C R V A V
ACCTTCTTCTCTACTTCTGGCTACCAACTACTACTGGATcCTGGTGGAGGGGCTGTAC
T F F L A Y F L A T N Y Y W I L V E G L Y
TTGCACAGCCTCATCTTACATGGCCTTTTTCTCAGAGAAGAAGTACCTGTGGGGCTTACCC
L H S L I F M A F F S E K K Y L W G F T
ATCTTTGGCTGGGGTCTACCGGCTGTCTTCTGCTGGCTGTGTGGGTGCGGTGTACAGCAACC
I F G W G L P A V F V A V W V G V R A T
TTGGCCAAACATGGGTGCTGGGATCTGAGCTCCGGGCACAAGAAGTGGATCATCCAGGTG
L A N T G C W D L S S G H K K W I I Q V
CCCATCCTGGCATCTGTTGTGCTCAACTTCATCCTTTTTATCAACATCATCCGGGTGCTT
P I L A S V V L N F I L F I N I I R V L
GCCACTAAGCTTCGGGAGACCAATGCGGGCGGTGTGACACCAGGCAGCAGTACCGGAAG
A T K L R E T N A G R C D T R Q Q Y R K
CTGCTCAGGTCCACGTTGGTGCTCGTGCCGCTCTTTGGTGTgCACTACACCGTCTTCATG
L L R S T L V L V P L F G V H Y T V F M
GCCTTGCCGTACACCGAGGTCTCAGGGACATTGTGGCAGATCCAGATGCATTATGAGATG
A L P Y T E V S G T L W Q I Q M H Y E M
CTCTTCAACTCCTTCCAGGGATTTTTTGTGTCATATATACTGTTTCTGCAATGGTGAG
L F N S F Q G F F V A I I Y C F C N G E
GTGCAGGCAGAGATTAGGAAGTCATGGAGCCGCTGGACACTGGCGTTGGACTTCAAGCGC
V Q A E I R K S W S R W T L A L D F K R
AAAGCACGAAGTGGGAGTAGCAGCTACAGCTATGGCCCAATGGTGTCTCACACGAGTGTG
K A R S G S S S Y S Y G P M V S H T S V
ACCAATGTGGGCCCCCGTGCAGGACTCAGCCTCCCCCTCAGCCCCCGCTGCCTCCTGCC
T N V G P R A G L S L P L S P R L P P A
ACTACCAATGGCCACTCCCAGCTGCCTGGCCATGCCAAGCCAGGGGCTCCAGCCACTGAG
T T N G H S Q L P G H A K P G A P A T E
ACTGAAACCCTACCAGTCACTATGGCGGTTCCCAAGGACGATGGATTTCCTTAACGGCTCC
T E T L P V T M A V P K D D G F L N G S
TGCTCAGGCCTGGATGAGGAGGCTCCGGGTCTGCGCGGCCGCTCCATTGTTGCAGGAA
C S G L D E E A S G S A R P P P L L Q E
GGATGGGAAACAGTCATGTGA SEQ ID No: 36
G W E T V M * SEQ ID No: 37

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Figure 8

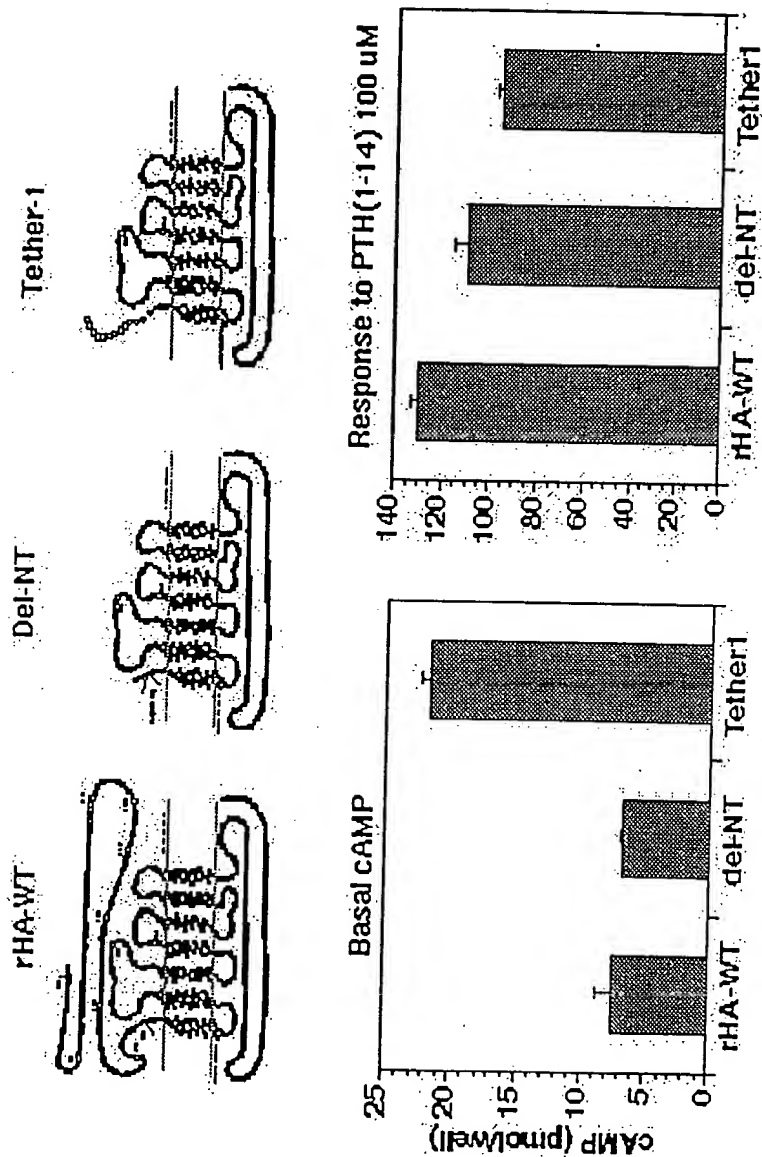


Figure 9

MAP of: Tether-1C, Seq check: 6795 from: 2319 to: 3326
Stop codon at 481 added to Tether-1

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a   ATGGGGGGCCGCCGGATGGCACCCAGCCTGGCGCTCCTACTCTGCTGCCAGTGCTCAGC
    M G A A R I A P S L A L L L C C P V L S -

a   TCCGCCcTATGCGGUUCCGAAAUCCAGCUGAUGCACggcggaggaggcGAGGTATTTGAC
    S A Y A V S E I Q L M H G G G G E V F D -

a   CGCCTAGGCATGATCTACACCGTGGGATACTCCATGTCTCTCGCCTCCCTCACGGTGGCT
    R L G M I Y T V G Y S M S L A S L T V A -

a   GTGCTCATCCTGGCCTATTTTAGGCGGCTGCAC TGACGCGCAACTACATCCACATGCAC
    V L I L A Y F R R L H C T R N Y I H M H -

a   ATGTTCTCTGTCGTTTATGCTGCGCGCGCGGAGCATCTTCGTGAAGGACGCTGTGCTCTAC
    M F L S F M L R A A S I F V K D A V L Y -

a   TCTGGCTTCACGCTGGATGAGGCCGAGCGCCTCACAGAGGAAGAGTTGCACATCATCGCG
    S G F T L D E A E R L T E E E L H I I A -

a   CAGGTGCCACCTCCGCCGGCCGCTGCCGCCGTAGGCTACGCTGGCTGCCCGGTGGCGGTG
    Q V P P P P A A A A V G Y A G C R V A V -

a   ACCTTCTTCTCTACTTCCCTGGCTACCAACTACTACTGGATcCTGGTGGAGGGGCTGTAC
    T F F L Y F L A T N Y Y W I L V E G L Y -

a   TTGCACAGCCTCATCTTCATGGCCTTTTTTCTCAGAGAAGAAGTACCTGTGGGGCTTCACC
    L H S L I F M A F F S E K K Y L W G F T -

a   ATCTTTGGCTGGGGTCTACCGGCTGTCTTCGTGGCTGTGTGGGTGGGTGTGAGAGCAACC
    I F G W G L P A V F V A V W V G V R A T -

a   TTGGCCAACTGCGGTGCTGGGATCTGAGCTCCGGGCACAAGAAGTGGATCATCCAGGTG
    L A N T G C W D L S S G H K K W I I Q V -

a   CCCATCCTGGCATCTGTTGTGCTCAACTTCATCCTTTTTATCAACATCATCCGGGTGCTT
    P I L A S V V L N F I L F I N I I R V L -

a   GCCACTAAGCTTCGGGAGACCAATGCGGGCCGGTGTGACACCAGGCAGCAGTACCGGAAG
    A T K L R E T N A G R C D T R Q Q Y R K -

a   CTGCTCAGGTCCACGTTGGTGTCTGCGCGCTCTTTGGTGTgCACTACACCGTCTTCATG
    L L R S T L V L V P L F G V H Y T V F M -

a   GCCTTGCCGTACACCGAGGTCTCAGGGACATTGTGGCAGATCCAGATGCATTATGAGATG
    A L P Y T E V S G T L W Q I Q M H Y E M -

a   CTCTTCAACTCCTTCCAGGGATTTTTTGTGTCATCATATACTGTTTCTGCAATGGTGAG
    L F N S F Q G F F V A I I Y C F C N G E -

a   GTGCAGGCAGAGATTAGGAAGTCATGGAGCCGCTGGACACTGGCGTaG SEQ ID NO:38
    V Q A E I R K S W S R W T L A * SEQ ID NO:39
  
```

Figure 10

MAP of rdel (Nt/Ct)

a ATGGGGGCGCCCGGATCGCAOCCAGCCTGGCGCTCCTACTCTGCTGCCAGTGCCTCAGC
 M G A A R I A P S L A L L L C C P V L S -
 a TCCGCATATGCGCTGGAGGTATTTGACCGCCTAGGCATGATCTACACCGTGGGATACTCC
 S A Y A L E V F D R L G M I Y T V G Y S -
 a ATGTCTCTCGCCTCCCTCACGGTGGCTGTGCTCATCCTGGCCTATTTTAGGCGGCTGCAC
 M S L A S L T V A V L I L A Y F R R L H -
 a TGCACGCGCAACTACATCCACATGCACATGTTCTGTCGTTATGCTGGCGCGCGGAGC
 C T R N Y I H M H M F L S F M L R A A S -
 a ATCTTCGTGAAGGAAGCTGTGCTCTACTCTGGCTTCACGCTGGATGAGGCCGAGCGCCTC
 I F V K D A V L Y S G F T L D E A E R L -
 a ACAGAGGAAGAGTTGCACATCATCGCGCAGGTGCCACCTCCGCCGCGCGCTGCCGCGTA
 T E E E L H I I A Q V P P P P A A A A V -
 a GGCTACGCTGGCTGCCGCGTGGCGGTGACCTTCTTCCTCTACTTCCTGGCTACCAACTAC
 G Y A G C R V A V T F F L Y F L A T N Y -
 a TACTGGATCTGGTGGAGGGGCTGTACTTGCACAGCCTCATCTTCATGGCCTTTTCTCA
 Y W I L V E G L Y L H S L I F M A F F S -
 a GAGAAGAAGTACCTGTGGGGCTTCACCATCTTTGGCTGGGGTCTACCGGCTGTCTTCGTG
 E K K Y L W G F T I F G W G L P A V F V -
 a GCTGTGTGGTGGTGTGAGCAACCTTGGCCAACACTGGGTGCTGGGATCTGAGCTCC
 A V W V G V R A T L A N T G C W D L S S -
 a GGGCACAAGAAGTGGATCATCCAGGTGCCCATCCTGGCATCTGTTGTGCTCAACTTCATC
 G H K K W I I Q V P I L A S V V L N F I -
 a CTTTTATCAACATCATCCGGGTGCTTGCCACTAAGCTTCGGGAGACCAATGCGGGCCGG
 L F I N I I R V L A T K L R E T N A G R -
 a TGTGACACCAGGCAGCAGTACCGGAAGCTGCTCAGGTCCACGTTGGTCTCGTCCCGCTC
 C D T R Q Q Y R K L L R S T L V L V P L -
 a TTTGGTGTGCACTACACCGTCTTCATGGCCTTGCCGTACACCGAGGTCTCAGGGACATTG
 F G V H Y T V F M A L P Y T E V S G T L -
 a TGGCAGATCCAGATGCATTATGAGATGCTCTTCACTCCTTCCAGGGATTTTGTGTC
 W Q I Q M H Y E M L F N S F Q G F F V A -
 a ATCATATACTGTTTCTGCAATGGTGGAGGTGCAGGCAGAGATTAGGAAGTCATGGAGCCGC
 I I Y C F C N G E V Q A E I R K S W S R -
 a TGGACACTGGCGT_{ag} SEQ ID NO: 40
 W T L A * SEQ ID NO: 41

FIGURES 11A-11D

Oligo is designed to join PTH(1-9) sequence to core of receptor using a Gly linker. Test for constitutive activation.
Insert immediately after predicted signal peptidase cleavage site (Tyr23, use Ala24 as Ala1 of PTH. Join to Glu-182, - boundary of exonC/H1.
KHA.WT map red - flanking homology

(1-40)

ATGGGGGGGGGGGGGATCGCACCCAGCCTGGCGCTCTACTCTGCTGCCAGTCTCTCAGC
M G A A R I A P S L A L L L C C P V L S -

SEQ ID NO: 46 and 53.

NDEI--

TCCGCATATGGCTGGTGGATCGGACGATGTCTTTACCAAAGAGGAACAGATTTTCCTG
S A Y A L V D A D D V F T K E E Q I F L -

FIG. 11 A

(161-200)

AACCGGACGTGGGGCAACTACACCGAGTGCCTCAAGTTCATGACCAATGAGACCGGGAA
553 N R T W A N Y S E C L K F M T N E T R E - 612
CGGAGGTATTTGACCGCCTAGGCATGATCTACACCGTGGGATACTCCATGTCTCTCCG
613 R E V F D R L G H I Y T V G Y S H S L A - 672

SEQ ID NO 47 and 54

Backtranslate PTH(1-9) Gly4.:

CCUGUUUCCGAAAUCCAGCUGAUGCACggcgaggagggc

SEQ ID NO 48

Insert PTH(2-9).Gly4 between A24 and E182, use about 30 nts for flanking homology
5' flank Hom - 33 nt, 3' flank Hom - 30 nt, total - 99 nts.

FIG 11 B

Oligo: Sequence ID#: E16631A1

CTCTGCTGCCAGTGTCTCAGCTCCGCGCTATCGGGTTTCCGAAATCCAGCTGATGCACggcgaggagggc
GAGGTATTTGACCGCCTAGGCATGATCTAC

SEQ ID NO 49

CCG check:

Map of: Y23E182G4PTH1-9.seq from: 1 to: 99

DNA sequence for making Tethered PTH ligand/receptor

Receptor - rat PTH1 Rec

Insert PTH(1-9)-Gly4 sequence between Tyr23 and Glu182.

Use Ala24 as codon 1 of PTH, flanking homology of ~30 nts

Takes out NDEI site at Ala 22 CCA->GCC

With 1 enzymes: NDEI

FIG. 11 C

CTCTGCTGCCAGTGTCTCAGCTCCGCGCTATCGGGTTTCCGAAATCCAGCTGATGCACgggc
L C C P V L S S A Y A V S E I Q L H H G -
ggaggaggcGAGGTATTTGACCGCCTAGGCATGATCTAC
G G G E V F D R L G H I Y -

SEQ ID NO: 50 and 55

FIG. 11 D

Helix II: -/rec-dna> more tether-1.map from: 2350 to: 2650

tethered PTH(1-9) to core receptor.

PTH(1-9) linked to Glu-182 of rat receptor.

Insert immediately after Tyr23 cleavage site.

ligo tether 1 spans 2358 to 2691.

Takes out NDEI at 2390 Ala-22).

FIG. 11.E

NdeI CA'TA_TG Cuts at: 1790 1790
Size: 5769

MAPSORT of: rDeleI-G.seq from: 1. to: 5736

***To be used as template SS DNA for Tether-1 OM.

With 1 enzymes: NDEI

NdeI CA'TA_TG

Cuts at: 1790 2384 1790
Size: 594 5142

(A-4c)

Oligo Mini-HA-1:

Sequence ID#: E16853A1

CTCTGCTGCCAGTGTCTCACTCCGCATATccctacgacgtccccgactacgcggcgaggaggcgAGGTATTTGACCGCCTAGGCATGATCTAC

SEQ ID NO 51

FIG 11.F

MAP of: mini-HA.seq from: 1 to: 96
Oligo sequence for adding HA Epitope tag to Headless rat PlR.
Insert 9 aa tag, YPYDVPDYA, between Tyr23 and Glu182, using
Tyr 23 as codon 1 of tag, and add 4 glys for spacer. Flanking homology of -30
nts. Takes out NDEI site at Ala 24 GCG->ccc (31-33).

With 1 enzymes: NDEI

1 CTCTGCTGCCAGTGTCTCACTCCGCATATccctacgacgtccccgactacgcggcgaggaggcgAGGTATTTGACCGCCTAGGCATGATCTAC 60

SEQ ID NO: 52 and 56

A L C C P V L S S A Y P Y D V P D Y A G G -

61 ggaggcgAGGTATTTGACCGCCTAGGCATGATCTAC 96

FIG 11.G

A G C E V F D R L G H I Y -

Enzymes that do not cut: NdeI

Fig 12

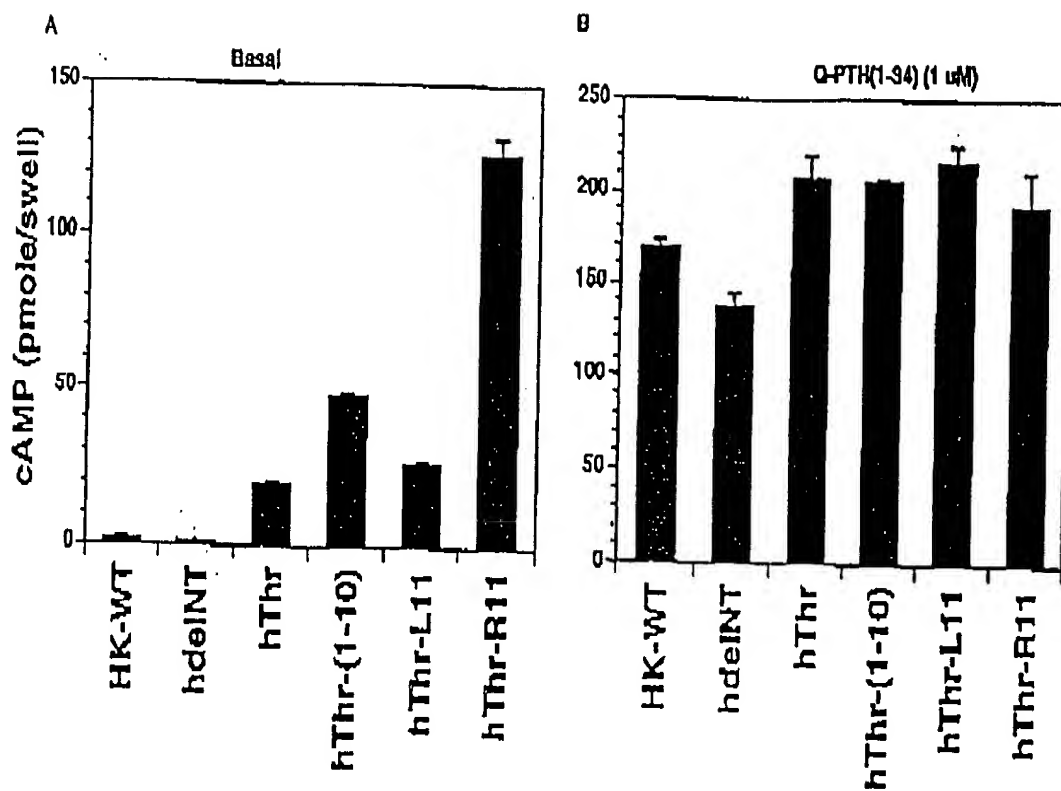


Fig 13

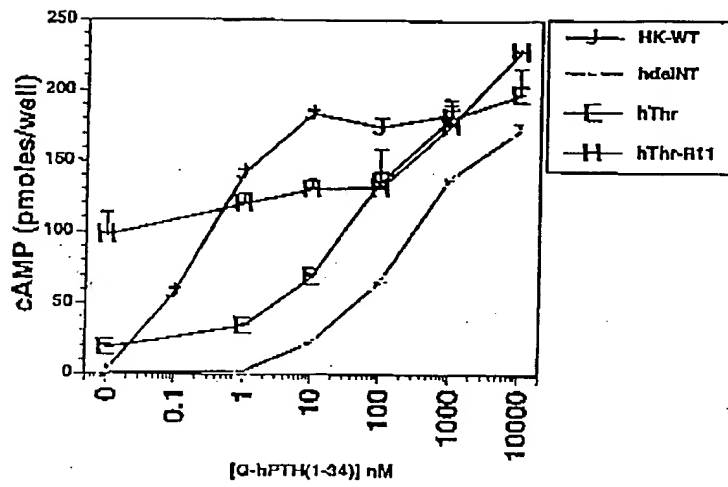


Fig 4

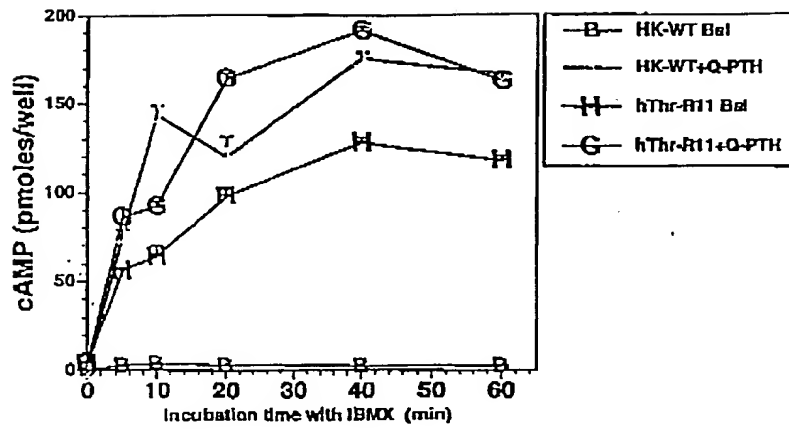


Fig 13

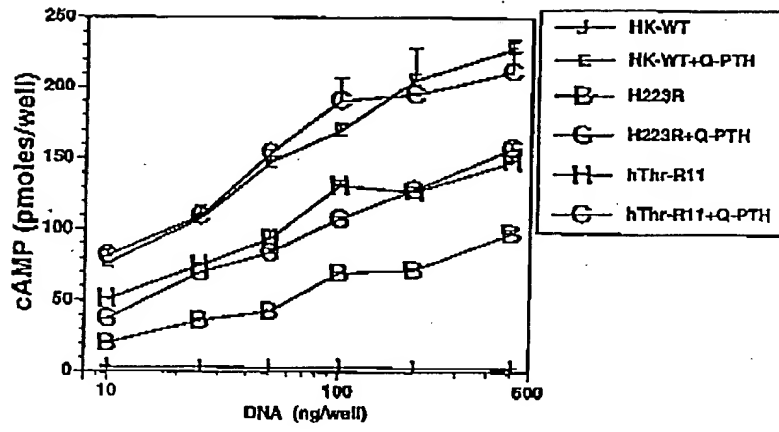


Fig 16

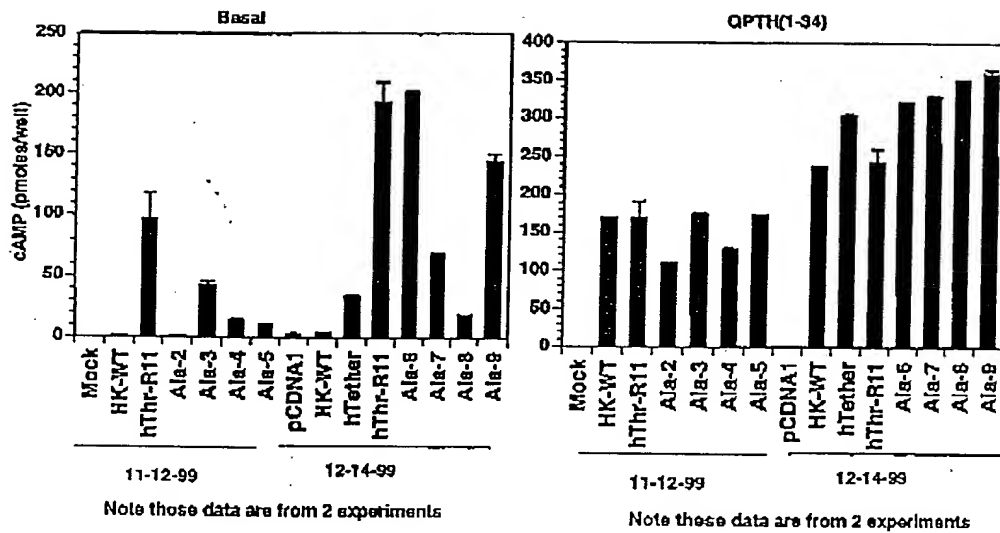


FIGURE 17

hTether-1 From human PTH-1 receptor by replacing Ala24 to Arg181 with Ala1 to His9 of PTH, then 4-Gly linker between His9 and Glu182 by oligonucleotide mutagenesis with oligo E20986

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2287 atggggAaccGccggatcgacacccggcctggcgctcctgctctgctgccccgtgctcagc
a M G T A R I A P G L A L L L C C P V L S 2346
2347 tccgcgtacgcgggtttccgaaatccagctgatgcacggcgaggaggogaggtgtttgac
a S A Y A V S E I Q L M H G G G G E V F D 2406
2407 cgcctgggcatgatttacaccgtgggtactcctgtcctctggcgtccctcaccgtagct
a R L G M I Y T V G Y S V S L A S L T V A 2466
2467 gtgctcactcctggcctactttagggcgctgcactgcacgcgcaactacatccacatgcac
a V L I L A Y F R R L H C T R N Y I R M H 2526
2527 ctgttctctgtccttcatgtcgcgcgcgtgagcatcttcgtcaaggacgctgtgtotao
a L F L S F M L R A V S I F V K D A V L Y 2586
2587 tctggcgccacgcttgatgaggctgagcgctcaccgaggaggagctgcgcgcacatgcc
a S G A T L D E A E R L T E E E L R A I A 2646
2647 caggcgcccccgccgctgccaccgcgctgcgcgctacgcgggtgcagggtggctgtg
a Q A P P P P A T A A A G Y A G C R V A V 2706
2707 accttcttcttcttacttcttggccaccaactactactggattctggtggagggtgtac
a T F F L Y F L A T N Y Y W I L V E G L Y 2766
2767 ctgcacagcctcatcttcatggccttcttctcagagaagaagtacgtggggcttcaca
a L H S L I F M A F F S E K K Y L W G F T 2826
2827 gtcttcgggtgggtctgcgcgctgtctctcgtggctgtgtgggtcagctgcagagctacc
a V F G W G L P A V F V A V W V S V R A T 2886
2887 ctggccaacacgggtgctgggacttgagctccgggaacaaaagtggatcatccagggtg
a L A N T G C W D L S S G N K K W I I Q V 2946
2947 cccatcctggcctccattgtgtcctaacttcatccttcatcaatatcgtccgggtgctc
a P I L A S I V L N F I L F I N I V R V L 3006
3007 gccaccaagctgcgggagaccaacgcggcggtgctgacacacggcagcagtagccggaag
a A T K L R E T N A G R C D T R Q Q Y R K 3066
3067 ctgctcaaatccacgctggtgctcatgccccctcttggcgctccactacattgtcttcacg
a L L K S T L V L M P L F G V H Y I V F M 3126
3127 gccacaccatacacggaggtctcaggagcgtctggcaagtcagatgcactatgagarg
a A T P Y T E V S G T L W Q V Q M H Y E M 3186
3187 ctcttcaactccttccagggttttttgcgaatcataactgtttctgcaatggcgag
a L F N S F Q G F F V A I I Y C F C N G E 3246
3247 gtacaagctgagatcaagaaatcttggagcgtggacactggcactggacttcaagcga
a V Q A E I K K S W S R W T L A L D F K R 3306
3307 aaggcagcagcgggagcagcagctatagctacggccccatggtgtccacacagtgctg
a K A R S G S S S Y S Y G P M V S H T S V 3366
3367 accaatgtggcccccggtgtggactcggcctgccctcagcccccgctactgccact
a T N V G P R V G L G L P L S P R L L P T 3426
3427 gccaccaccaacggccaccctcagctgcctggccatgccaaagccagggaccccgccctg
a A T T N G H P Q L P G H A K P G T P A L 3486
3487 gagaccctcgagaccacaccctgccatggctgctcccaaggacgatgggttctcaac
a E T L E T T P P A M A A P K D D G F L N 3546
3547 ggctcctgtcaggcctggagcaggagcctctgggcctgagcggccacctgccctgcta
a G S C S G L D E E A S G P E R P P A L L 3606
3607 cagggaagtgaggagacagtcatgtgaccaggcgctgggggct
a Q E E W E T V M 3649

```

SEQ ID NO: 61
SEQ ID NO: 62

FIGURE 18

hdeLNT

human PTE-1 rec deleted for 24-181; Joins Tyr23 to Glu182.
February 25, 1999 13:38 ..

SEQ 10 NO: 59.

SEQ ID NO: 60

[illegible]

FIGURE 19

hTether-R11

Made from hTether-1 by inserting Asn10-Arg11 between His9 and first Gly of linker by oligonucleotide mutagenesis with Oligo - E27309

	atgggGACCgCccggatcgcaaccggcgctggcgctcctgctctgctgccccgctgctcagc	SEQ ID NO: 57
a	2287 M G T A R I A P G L A L L L C C P V L S 2346	SEQ ID NO: 58
	tcgcgctacgcgggtttccgaaatccagctgatgcaTAATCGTggcgaggaggcgaggtg	
a	2347 S A Y A V S E I Q L M H N R G G G G E V 2406	
	tttgaccgctgggcatgatttacaccgtgggctactccgtgctccctggcgctccctcacc	
a	2407 F D R L G M I Y T V G Y S V S L A S L T 2466	
	gtagctgtgctcatcctggcctacttttagcggtgcaactgcacgcgaactacatccac	
a	2467 V A V L I L A Y F R R L H C T R N Y I H 2526	
	atgcaccgctcctgtccttcctgctgctgcgcgcctgagcatcttcgtcaaggacgctgtg	
a	2527 M H L F L S F M L R A V S I F V K D A V 2586	
	ctctactctggcgccacgcttgatgaggtgagcgctcaccgaggaggagctgcgcgc	
a	2587 L Y S G A T L D E A E R L T E E E L R A 2646	
	atcgccaggcgccccgcgcgctgccaacgcgcgtgcgcgtacgcgggtgcagggtg	
a	2647 I A Q A P P P P A T A A A G Y A G C R V 2706	
	gctgtgaccttcttcttacttctgcccaccaactactactgattctggtggagggg	
a	2707 A V T F F L Y F L A T N Y Y W I L V E G 2766	
	ctgtaccgcacagcctcatcttcatggcctcttctcagagaagaagtacctgtggggc	
a	2767 L Y L H S L I F M A F F S E K K Y L W G 2826	
	ttcacagtcttcggtgggtctgcccgtgtcttctggtgtgtgggtcagtgctaga	
a	2827 F T V F G W G L P A V F V A V W V S V R 2886	
	gctaccctggccacacgggtgctgggacttgagctccgggaacaaaaagtggatcacc	
a	2887 A T L A N T G C W D L S S G N K K W I I 2946	
	cagggtgcccatcctggcctccattgtgctcaacttcctcttcatcaatcctgctcgg	
a	2947 Q V P I L A S I V L N F I L F I N I V R 3006	
	gtgctcgccaccaagctgcgggagaccaacgcggcggtgtgacacacggcagcagtac	
a	3007 V L A T K L R E T N A G R C D T R Q Q Y 3066	
	cggagctgctcaaatccacgctggtgctcatgccctcttggcgctccactacattgtc	
a	3067 R K L L K S T L V L M P L F G V H Y I V 3126	
	ttcatggccacaccatacaccgaggtctcaggagcgtctggcaagtccagatgcactat	
a	3127 F M A T P Y T E V S G T L W Q V Q M H Y 3186	
	gagatgctcttcaaotccttccagggtatrrttgtgcgaatcatatactgtttctgcaat	
a	3187 E M L F N S F Q G F F V A I I Y C F C N 3246	
	ggcgaggtacaagctgagatcaagaaatcttggagccgctggacactggcactggacttc	
a	3247 G E V Q A E I K K S W S R W T L A L D F 3306	
	aagcgaaaggcagcagcgggagcagcagctatagctacggccccatggtgtccacaca	
a	3307 K R K A R S G S S S Y S Y G P M V S H T 3366	
	agtgtgaccaatgtcggccccgtgtgggactcggcctgccccacagccccgcctactg	
a	3367 S V T N V G P R V G L G L P L S P R L L 3426	
	ccactgccaccaccaacggccaccctcagctgcccggccatgccaaagcagggaaccca	
a	3427 P T A T T N G H P Q L P G H A K P G T P 3486	
	gccctggagaccctcgagaccacaccctgccatggctgctcccaaggacgatgggttc	
a	3487 A L E T L E T T P P A M A A P K D D G F 3546	
	ctcaacggtcctgctcaggcctggacgaggaggcctctgggctgagcgccactgccc	
a	3547 L N G S C S G L D E E A S G P E R P P A 3606	
	ctgctacaggaagagtgaggagacagtcattgtgaccaggcgctgggggctggacctgcta	
a	3607 L L Q E E W E T V M * 3666	